LABORATORY ANIMAL CARE

CROSS INFECTION WITH EIGHTEEN PATHOGENS AMONG CAGED LABORATORY ANIMALS[1,2,3,4]

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SUMMARY • *The extent of cross infection among caged laboratory monkeys, guinea pigs, mice, and chickens was examined with 18 pathogens. Experiments were designed to determine if cross infection would be eliminated by 1) ventilated cages, 2) various air-washing procedures, 3) elimination of excreta from cages, 4) ultraviolet irradiation, and/or 5) high efficiency air filters covering the cages. Animals exposed to an aerosol create a hazard to the experiment and experimenter because the usual post-exposure air-washing techniques do not remove microorganisms entrained on the fur. A forceful air-jet ruffling technique greatly reduced microorganisms from the fur. Ultraviolet irradiation or high efficiency air filters on the cages prevented infection of normal animals in adjacent cages. With most microorganisms, animals inoculated other than by aerosol challenge do not show cross infection.*

Cross infection among experimental animals, with its potential effect on the experiment, and the implied threat of transmission of disease to the animal handler, becomes more important as the need for animals in scientific research continues to increase. Primate centers have been established to provide information on the care and handling of nonhuman primates so that investigators can develop and maintain healthy animals for many purposes, including research on cancer, malaria, tuberculosis, and other diseases. New species (bats, hedgehogs, gerbils, marmosets, etc.) have been introduced in the laboratory, and specific-pathogen-free animals are used extensively. When experimental animals are held for several months or years, there always is danger of losing these increasingly valuable animals from nonspecific infection. During short-term experiments with infectious microorganisms, there sometimes is danger of transmission of infection to normal control animals or to the animal caretaker.

The danger to the animal caretaker has long been emphasized. In 1951 the Expert Group on Zoonoses was established under the joint auspices of the World Health Organization and the Food and Agriculture Organization, and prepared a list of zoonoses (**30**). Sulkin and Pike (**26**) reported that 10.3% of 1,342 laboratory infections resulted from handling infected animals or ectoparasites. Wedum (**27**) reported 12% of animal caretakers had been infected, compared to 21% and 18% of trained scientific personnel and laboratory technical assistants, respectively.

One sure indicator of danger to personnel is infection of normal animals by their inoculated cagemates. Lurie (**16**) presented data of cross infection occurring in normal guinea pigs housed in the same room with tuberculous guinea pigs. Kirchheimer *et al* (**12**) reviewed the literature and reported cross infection occurring with 12 microorganisms.

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[1] From the Industrial Health & Safety Directorate, Fort Detrick, Frederick, Maryland 21701.

[2] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences — National Research Council.

[3] The technical assistance and critique of Dr. Peter J. Gerone and LTC Robert W. McKinney are gratefully acknowledged. We are indebted to the contributions of personnel mentioned in the footnotes throughout the text; to Mr. Theron D. Green and Mr. Wayne D. Leeder for their assistance in many monkey experiments; to Drs. Garrett V. Keefer, William P. Allen, Henry T. Eigelsbach, William T. Roessler, Richard F. Berendt, Maurice L. Gus, and Charles W. Beard for supplying microorganisms; to Robert D. Boyer for fabricating plastic hoods; and finally to the highly competent animal caretaker, Mr. Russell A. Thomas, whose care and assistance in the animal experiments made this study possible.

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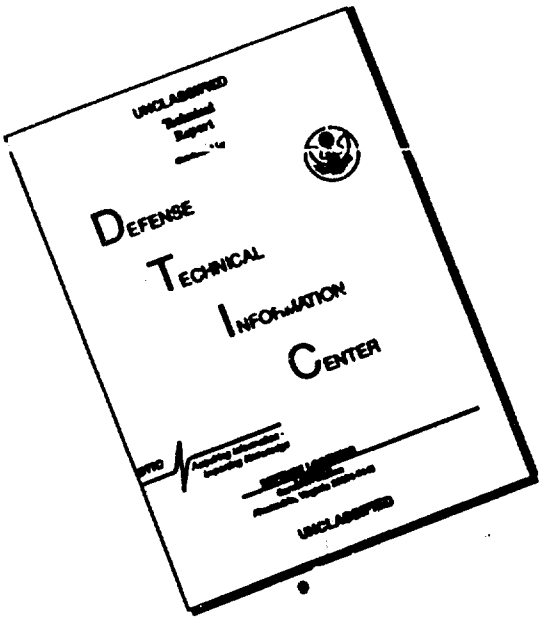

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An extensive review of the literature supplemented information compiled by Wedum (27), who reported cross infection occurring with 19 microorganisms, and Wedum and Kruse (28) who reported cross infection occurring with 77 microorganisms. Analysis revealed that: 1) 63% of the 169 references on cross infection were reported within the past decade, and 87% within the last 20 years; 2) special caging methods were desirable when aerosol-exposed animals were caged, because cross infection occurred frequently; and 3) cross infection was less among parenterally injected animals than in aerosol-exposed animals. A subsequent laboratory program was designed to supply data on special caging methods for representative significant human pathogens. One of these methods is the use of the closed ventilated cage for primates (11).

The objectives of the present experiments were to determine whether 1) ventilated cages prevent infection of normal cagemates and remove the implied danger to the animal caretaker from animals exposed to aerosols of highly infectious microorganisms; 2) cagemate infection could be prevented by variations in the post-exposure air-washing technique; 3) infection of normal cagemates would occur when monkeys were kept in open wire cages after parenteral injections; and 4) post-exposure air-washing of animals to prevent transmission of disease between adjoining cages could be eliminated if other caging methods were used, such as a wire cage that allowed urine and feces to drop from the cage, ultraviolet irradiation of the air above the cage, or high efficiency air filters that cover the top of the cage.

The first of these studies has been reported (14) in which monkeys, whose bodies were exposed to aerosols of *Coccidioides immitis* arthrospores, infected cagemates despite various air-washing procedures, but in which forceful ruffling of the fur by manipulation of an air hose did clean the fur to a point where no cagemates were infected. These experiments define a clear differentiation between *cross infection*, in which diseased animals infect cagemates by urine, feces, saliva, or droplets, and *cross contamination*, in which cagemates are infected by organisms released from the fur or skin before the exposed animals become ill.

The present report extends the work, begun with *C. immitis*, to other infectious microorganisms, and describes procedures used for data presented by Wedum and Kruse (28).

Materials and Methods

Experimental animals: Monkeys (*Macaca mulatta*) of both sexes, weighing from 1.1–4.3 kg; guinea pigs of the Hartley strain weighing from 0.2–0.4 kg; mice (FD(SW) Rockefeller Institute 1949) weighing from 8–14 g; and White Leghorn chickens, 1, 2, and 3 weeks old, were employed in these studies. The animals were observed daily and fed water and Purina monkey chow (Ralston Purina Co., St. Louis, Mo.), Rockland rat and mouse pellets, Rockland guinea pig pellets (Teklad Inc., Winfield, Iowa), and chicken growing feed (Sherwood Feed Co., Baltimore, Md.) *ad libitum*. When necessary, rectal temperatures were determined by a thermistor probe (Tri-R-Instrument Co., Jamaica, N.Y.).

Animal inoculation: Animals were inoculated either by the respiratory route, by intranasal (i.n.) or intratracheal (i.t.) instillation, or by intracerebral (i.c.), intramuscular (i.m.), intraperitoneal (i.p.), intravenous (i.v.) or subcutaneous (s.c.) injection. Monkeys were tranquilized by i.m. injection (0.1 mg/kg body weight) of phencyclidine hydrochloride (Sernylan®, Parke, Davis & Co., Detroit, Mich.).

Respiratory exposures took place in an aerosol chamber within a gastight ventilated cabinet (14). All aerosols were created by a Vaponefrin nebulizer (Vaponefrin Co., Edison, N.J.) with 1 exception; the fungal spores were aerosolized by compressed air. The whole body or only the head of the animal was exposed to the microbial aerosol.

All-glass impingers (AGI-30) (29) were operated throughout each exposure, and the viable concentrations ascertained by assaying serial 10-fold dilutions. If a bacterium or fungus was the test organism, samples from the serial 10-fold dilutions were plated on an agar medium specific for the microorganism. If a virus, rickettsia, or psittacosis agent was the test organism, samples from the serial 10-fold dilutions were inoculated into animals or tubes of monolayer tissue culture. The inhaled doses were calculated from the data on aerosol concentration determined by assay of AGI-30 fluids and from the respiratory minute volumes. For monkeys the calculations from the formula of Berendt (1) were used, and for guinea pigs or mice calculations from the formula of Guyton (8) were used.

For i.n instillation, guinea pigs or mice were anesthetized with ether before instilling 0.2 ml and 0.05 ml, respectively, of the microbial suspension with a needle and syringe into the nares. After inoculation, the nasal area was wiped with a cotton pledget moistened with a disinfectant appropriate for the particular microorganism.

Chickens were inoculated i.t. by instilling 0.1 ml of the microbial suspension with a blunt needle and syringe into the trachea. After inoculation, the head was wiped with a disinfectant.

Parenteral injections were done in the gastight, ventilated cabinet. A cotton pledget moistened with disinfectant appropriate for the particular microorganism was used to disinfect each injection site before and after inoculation and to surround the needle of the hypodermic syringe, so as to prevent contamination by infectious fluid or accidental microbial aerosol.

Air-washing: Each inoculated animal was placed in the transfer cabinet and air-washed to reduce contamination of the fur (Fig. 1). Various methods were used for monkeys:

A) The monkey was placed in the attached transfer cabinet through which filtered air flowed at 150 l/min. After 15 min of this air-wash, the monkey was moved from the transfer cabinet to an attached, closed ventilated cage;

B) The monkey was placed in the transfer cabinet, and a removable flexible line and nozzle were attached to the air intake line within the transfer cabinet. Air flow was maintained at 150 l/min of filtered air, but was directed through the nozzle at the monkey to ruffle the fur. The monkey was manipulated so that all parts of the body were air-washed for 10 min by this forceful jet of air. The line was then removed, and the usual air flow was continued for 5 more min, at which time the monkey was shifted from the transfer cabinet to an attached, closed ventilated cage;

C) Same as method A except the air-wash continued for 25 min;

D) Same as method A except air-wash continued for 10 min. After the air-wash, a towel moistened with 2% quaternary ammonium compound was moved into the transfer cabinet, and the monkey was wiped with this towel.

Guinea pigs and mice were air-washed as monkeys in method A. After air-washing, animals were transferred to the animal room (*see Caging of Animals*). Before other animals were air-washed, the transfer cabinet was disinfected with a disinfectant specific for the particular microorganism. Then the transfer cabinet was rinsed with a neutralizer specific for the disinfectant. After 5 min contact time, the cabinet was washed with water.

CAGING OF ANIMALS

Monkeys: A) Cage contained an unexposed monkey (cagemate control) when attached to the transfer cabinet of the gastight, ventilated cabinet. After the exposed monkey was placed in the cage, the closed space connecting the transfer cabinet to the cage was disinfected. The cage with the 2 monkeys was detached from the transfer cabinet and transported to the animal room. There it was connected by a 3-ft-long air

Fig. 1. *Air-washing monkey in transfer cabinet. U. S. Army photograph.*

duct to a 2nd closed, ventilated cage that housed another unexposed control monkey (Fig. 2). Air passed through these cages at 65 l/min. Airflow was from the room, through a high-efficiency filter (5) into the first cage housing the aerosol-exposed monkey and his cagemate control, then out of this cage through a short rubber air duct into and through the 2nd cage housing the 2nd control monkey, into a manifold that contained an ultrahigh-efficiency filter, and finally to the exhaust plenum. Air-sampling ports were located in the air duct that connected the first cage, containing the aerosol-exposed monkey and a cagemate control, and the 2nd cage; and in the exhaust air duct from the 2nd cage housing a control monkey. After exposure at various time intervals, 10 cu ft of air were sampled from each port either by a funneled sieve sampler (7) containing an agar plate, or by an AGI-30 containing liquid medium. Assay for microorganisms was done by incubating the agar plates or by inoculating appropriate animals.

B) The cage attached to the transfer cabinet did not contain a cagemate control. After disinfection, the cage containing only the exposed monkey was detached and transferred to the animal room. The air duct was connected to the manifold; an air-sampling port was located in the exhaust air duct. No adjoining cages were used. At 24-hr intervals after microbial exposure, non-exposed cagemate control monkeys were placed in the cages housing exposed monkeys.

C) Parenterally injected monkeys were individually air-washed in the transfer cabinet for 15 min and then moved to an animal-carrying container by which they were transported to the animal room. Here they were placed in open wire cages with an uninoculated cagemate control.

D) Monkeys used in *Herpesvirus simiae* Monkey B experiments were air-washed, placed in a plastic bag, transported to the

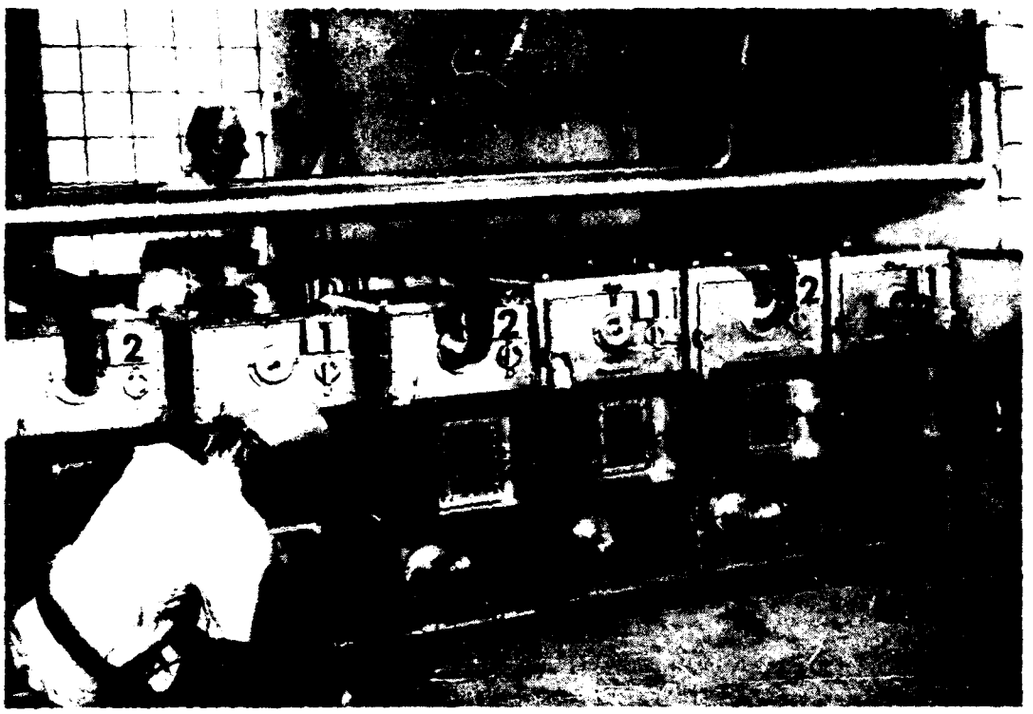

Fig. 2. *Ventilated cages for aerosol-exposed monkeys. U. S. Army photograph.*

animal section of the gastight, ventilated cabinet, and placed in a restraining chair. The system simulated the housing of monkeys in closed ventilated cages. A "cagemate control" was in a restraining chair 5 inches from the exposed monkey. An "adjacent cage control" was placed in a restraining chair contained within a plastic cabinet constructed so that air from the area caging the exposed and cagemate control was drawn through the plastic cabinet at 65 l/min, into a duct, through a filter, and into the exhaust manifold.

Guinea pigs: Guinea pigs were housed in cages with solid sides and bottoms, and louvered lids. Cage dimensions were 21.5" x 10" x 9". The cage lid contained openings for a feeder and long-stemmed water bottles that allowed feeding and watering the animals without removing the lid. Ab-Sorb-Dri® (Ab-Sorb-Dri, Inc., Garfield, N.J. was used for cage litter with guinea pigs, mice, and chickens. Cages were placed on racks with 4 shelves, each holding 4 cages.

A) Inoculated guinea pigs and uninoculated guinea pigs (controls) were caged in the following ratios: 1) 1 control to 2 inoculated; 2) 2 controls to 1 inoculated; 3) 2 controls to 2 inoculated; and 4) 3 controls 0.5 inches from a cage with 3 inoculated.

B) A heavy wire screen 1.5 inches above the bottom of the cage allowed the animals' urine and feces to drop through the screen to the litter, so that the guinea pigs did not walk in the cage litter.

C) Ultraviolet lamps were situated above each shelf of the cage rack so that ultraviolet irradiation with an average intensity of 350 μ watts/sq cm was emitted across the tops of the animal cages, but did not penetrate down into the cages where the animals were located.

D) A 0.5-inch thick high efficiency air filter (50 FG) was attached to the cage lid.

Mice: A Mice were housed in cages 8" x 4" x 4". The cages were constructed with

3 solid sides and with the front and bottom made of wire mesh that allowed urine and feces to drop from the cage onto collecting material. In the experiments, mice caging ratios were: 1) 1 control to 4 inoculated; 2) 2 controls to 3 inoculated; and 3) 5 controls 1.5 inches from a cage with 5 inoculated mice.

B) Mice were housed in cages similar to guinea pigs, with solid sides and bottoms, and louvered lids, but cage dimensions were 10.75" x 8" x 6". Mice caging ratios were: 1) 1 control to 4 inoculated; 2) 2 controls to 3 inoculated; 3) 3 controls to 2 inoculated; and 4) 5 controls 2.0 inches from a cage with 5 inoculated mice.

C) A heavy wire screen 1.5 inches above the bottom of the cages (described in B) allowed the urine and feces to drop to the litter, so that the mice did not walk in the cage litter.

D) Ultraviolet lamps were situated above each shelf of the cage rack so that ultraviolet irradiation (350 μ watts/sq cm) was emitted across the tops of the animal cages described in B.

Chickens: Chickens were caged as were guinea pigs. However, an automatic watering system using Hart's watering cups (H. W. Hart Co., Glendale, Calif.) fed by gravity flow was installed in the cages, and chickens were fed granular growing feed. The cage lids were not removed for feeding and watering during the experiment. Cages were placed on racks with 4 shelves, each holding 4 cages.

A) Inoculated chickens and uninoculated chickens were caged in the following ratios: 1) 1 control to 4 inoculated; 2) 2 controls to 3 inoculated; 3) 3 controls to 2 inoculated; 4) 5 controls 0.5 inches from a cage with 5 injected chickens; and 5) no cagemate control chickens were used, only adjacent cage controls.

B) Wire partitions were added to a number of cages, thereby housing the chickens on wire that allowed feces to drop through to the litter, so that the chickens could not scratch in litter.

C) Ultraviolet lamps were situated above each shelf of the cage rack so that ultraviolet irradiation (350 μ watts/sq cm) was emitted across the tops of the cages. A 0.5-inch thick high efficiency air filter (50 FG) was attached to each cage lid.

D) Only ultraviolet irradiation was used.

E) Only the 50 FG air filters were used.

MICROORGANISMS

These are listed in Table 1. The table summarizes all data relating to microorganisms and animals used, inoculating routes, the size of the inoculum, air-washing and caging procedures, and the method by which infection, not only of inoculated animals but also controls, was confirmed. This tabular method is used to provide the reader with a quick review of the extent of the experiments.

ASSAY PROCEDURES

Bacteria: A) Bacteremia was verified by collecting blood from the monkeys before inoculation and daily after inoculation. Heparinized samples of blood (1,000 U.S.P. units/ml of sodium heparin (The Upjohn Co., Kalamazoo, Mich.) were inoculated into liquid media and incubated. Samples from the liquid media were examined microscopically and streaked on solid media. After 21 days, samples of negative blood cultures were injected i.p. into mice and guinea pigs for further confirmation.

B) An agglutination test verified tularemia infection. Monkeys and guinea pigs were bled before inoculation and at the conclusion of the experiment for paired sera. A formalin-treated *Pasteurella tularensis* suspension received from Dr. H. Eigelsbach was used in the standard agglutination test (19).

C) Necropsies were performed on test animals that died during the experiment and upon survivors sacrificed at the conclusion of the experiment. Samples of tissue (heart, liver, lung, spleen, and respiratory tract

TABLE 1

Summary of inoculation procedures, caging, and infection criteria

Disease or Microorganism	Strain	Hosts	Inoculation Routes[a]	Dose[b]	Method of Air-Washing[c]	Caging of Animals[d]	Criteria for Infection
Bacillus anthracis	V-1b	Monkeys	w.b., h.o.	1.1 x 10^3 spores	A, B, C	1A, 1B	Bacteremia
			i.m., i.p., i.v., s.c.	[e]	—	1C	Necropsy
		Guinea pigs	i.m., s.c.	1 x 10^3 spores	—	2A, 2B	
Brucella suis	FD	Monkeys	w.b., h.o.	1.35 x 10^3 cells	A, C	1A	Bacteremia
			i.m., i.p., i.v., s.c.	1 x 10^3 cells	—	1C	Necropsy
		Mice	i.m., i.n., i.p., s.c.	450 cells	—	3B	
Coxiella burneti	AD	Monkeys	w.b., h.o.	200 $GIPID_{50}$	A, B	1A	CF, Agglutination
			i.m., i.p., i.v., s.c.	200 $GIPID_{50}$	—	1C	Rickettsemia
		Guinea pigs	h.o.	209 $GIPID_{50}$	A	2A, 2C, 2D	
			i.m., i.n., i.p., s.c.	214 $GIPID_{50}$	—	2A	
Herpesvirus simiae	O'Hara	Monkeys	w.b., h.o.	4 x 10^4 $TCID_{50}$	A, B	1D	SN
Histoplasma capsulatum	3021	Monkeys	w.b., h.o.	550 spores	A, B, C	1A, 1B	Radiographs, CF
			i.m., i.p., i.v., s.c.	[f]	—	1C	Skin Test
		Guinea pigs	w.b.	420 spores	A	2A, 2B	Precipitins
			i.m., i.p., s.c.	210 spores	—	2A, 2B	Necropsy
		Mice	i.m., i.n., i.p., s.c.	207 spores	—	3B	
Influenza	PR8	Mice	h.o.	122 $MINLD_{50}$	A	3B	HI
			i.m., i.n., i.p., s.c.	110 $MINLD_{50}$	—	3B	
Japanese B encephalitis	Nakayama	Monkeys	w.b., h.o.	1 x 10^4 $MICLD_{50}$	A, B	1A	Viremia
		Mice	w.b., h.o.	210 $MICLD_{50}$	A	3B	SN
Mycobacterium tuberculosis	MSS-2180	Monkeys	w.b., h.o.	5 x 10^3 cells	A, B	1A	Radiographs
		Guinea pigs	h.o.	3.1 x 10^3 cells	A	2A, 2C, 2D	Skin Test
		Mice	w.b., h.o.	2.1 x 10^3 cells	A	3B, 3C	
			i.m., i.p.	2.5 x 10^3 cells	—	3B	
Newcastle disease virus	GB	Chickens	i.t.	5.1 x 10^3 ELD_{50}	—	4	HI
Pasteurella pestis	Kim-10	Guinea pigs	i.m., i.n., i.p.	980 cells	—	2A	Necropsy
		Mice	w.b.	986 cells	A	3B	
			i.m., i.n., i.p.	980 cells	—	3B	

TABLE 1 (*Continued*)

Disease or Microorganism	Strain	Hosts	Inoculation Routes[a]	Dose[b]	Method of Air-Washing[c]	Caging of Animals[d]	Criteria for Infection
Pasteurella tularensis	SCHU S4	Monkeys	w.b., h.o.	75 cells	A, B	1A	Bacteremia
			i.m., i.p., i.v., s.c.	75 cells	—	1C	Agglutination
		Guinea pigs	i.m., i.n.	81 cells	—	2A	Necropsy
Polio virus	Mahoney	Monkeys	w.b., h.o.	1×10^4 $TCID_{50}$	A, B	1A	Viremia
			i.m., i.p., i.v., s.c.	1×10^4 $TCID_{50}$	—	1C	SN
Psittacosis	Borg	Monkeys	w.b., h.o.	5×10^3 $MICLD_{50}$	A, B	1A	Septicemia
			i.m., i.p., i.v., s.c.	5×10^3 $MICLD_{50}$	—	1C	Radiographs
		Mice	i.c., i.p.	1×10^3 $MICLD_{50}$	—	3A	CF
Rickettsia rickettsi	Bitter Root	Monkeys	w.b., h.o.	670 $YSLD_{50}$	A, B, C	1A, 1B	Rickettsemia
			i.m., i.p., i.v., s.c.	69 $YSLD_{50}$	—	1C	CF
		Guinea pigs	i.p., s.c.	147 $YSLD_{50}$	—	2A	Necrosis
Rift Valley fever	Van Wyk	Monkeys	w.b., h.o.	5×10^2 $MICLD_{50}$	A, B	1A, 1B	Viremia
			i.m., i.p., i.v., s.c.	105 $MICLD_{50}$	—	1C	SN
		Mice	i.c., i.p.	217 $MICLD_{50}$	—	3A, 3B	Necropsy
St. Louis encephalitis	Hubbard	Monkeys	i.m., i.p., i.v., s.c.	1×10^3 $MICLD_{50}$	—	1C	Viremia, SN
		Mice	i.c., i.p.	496 $MICLD_{50}$	—	3A, 3B	Necropsy
Venezuelan equine encephalitis	Trinidad	Monkeys	w.b., h.o.	2.5×10^3 $MICLD_{50}$	A, B, D	1A, 1B	Viremia
			i.m., i.p., i.v., s.c.	1×10^3 $MICLD_{50}$	—	1C	SN
		Guinea pigs	h.o.	2.5×10^3 $MICLD_{50}$	A	2A	Necropsy
			i.m., i.p.	9.4×10^2 $MICLD_{50}$	—	2A, 2B	
		Mice	w.b.	1.5×10^3 $MICLD_{50}$	A	3B	
			i.c., i.p.	50 $MICLD_{50}$	—	3A, 3B	
Yellow fever	Asibi	Monkeys	w.b., h.o.	1×10^3 $MICLD_{50}$	A, B	1A	Viremia
			i.m., i.p., i.v., s.c.	1×10^3 $MICLD_{50}$	—	1C	SN
		Mice	i.c., i.p.	510 $MICLD_{50}$	—	3A, 3B	Necropsy

a. w.b. = whole body exposure; h.o. = head only exposure; i.c. = intracerebral; i.m. = intramuscular; i.n. = intranasal; i.p. = intraperitoneal; i.t. = intratracheal; i.v. = intravenous; s.c. = subcutaneous.

b. $GIPID_{50}$ = guinea pig intraperitoneal median dose (infects 50% of the animals); $TCID_{50}$ = tissue culture median infectious dose; $MINLD_{50}$ = mouse intranasal median lethal dose; $MICLD_{50}$ = mouse intracerebral median lethal dose; ELD_{50} = egg median lethal dose; $YSLD_{50}$ = yolk-sac median lethal dose.

c. See section entitled Air-Washing.

d. See section entitled Caging of Animals.

e. One monkey received 25 spores and 2 monkeys received 2.5×10^3 spores for each inoculation route.

f. One monkey received 50 spores and 2 monkeys received 255 spores for each inoculation route.

TABLE 2

Diluents, media, and incubation temperatures and times for confirmation of bacterial infection

Bacterium	Diluent	Plating medium	Incubation	
			°C	Days
Bacillus anthracis	Heart infusion broth[a]	2% blood agar	37	7
Brucella suis	1% Tryptose[a]—0.5% NaCl	Tryptose agar[a]	35	10
Mycobacterium tuberculosis	Dubos broth base[a]	Lowenstein-Jensen[a] Petragnani[a] Middlebrook 7H10[a] Dubos oleic acid[a]	37	42
Pasteurella pestis	Heart infusion broth[a]	Blood agar base[a,b]	28	10
Pasteurella tularensis	1% gelatin in 0.85% NaCl	Glucose cystine blood agar[c]	37	7

[a] Difco Labs., Detroit, Michigan.

[b] Containing 0.03% ferrous sulfate and 2.5 x 10^4 mg/ml vitamin B_1.

[c] Downs *et al* (6).

nodes) were weighed, placed in Ten Broeck tissue grinders with appropriate diluent, triturated, suspensions plated on suitable media, and incubated as shown in Table 2.

D) In the studies on tuberculosis, monkeys had radiographs[5] and skin tests with 25 mg (0.1 ml) Koch's Old Tuberculin (Jensen-Salsbery Laboratories, Kansas City, Mo.) inoculated intrapalpebrally and intradermally on the abdomen at 2-week intervals after inoculation. Guinea pigs were skin tested in the abdominal region before inoculation and at 2-week intervals after inoculation.

Fungi: A) Before inoculation 1) negative histoplasmin sensitivity was verified by injecting 0.1 ml histoplasmin intrapalpebrally in each monkey, and intradermally on the abdominal region of each guinea pig; 2) monkeys had radiographically clear lungs;[6] and 3) blood was withdrawn from each monkey and guinea pig and from a random sample of mice for sera to detect precipitins and CF antibodies.[7]

B) After inoculation 1) at 2-week intervals histoplasmin sensitivity was determined in monkeys and guinea pigs, and radiographs and sera studies were performed on monkeys; and 2) all animals were bled at the conclusion of the experiments for sera studies.

C) Complete necropsies were performed on all animals. Samples of tissue were removed aseptically from the lungs, the spleen, the liver, and the heart and examined microscopically for the yeast phase of *Histoplasma capsulatum*. Sections were triturated in 5 ml broth containing 1% Phytone (BBL) and 1% dextrose, and suspensions were plated on modified Sabouraud's agar containing 0.5 mg cycloheximide, 100 units penicillin, and 125 μg streptomycin per ml, and incubated at 30°C. All plates were kept 21 days before being discarded as negative for *H. capsulatum*.

Rickettsiae: A) When *Coxiella burneti* was the test rickettsia, monkeys were bled before inoculation and at 2-day intervals 5–15 days after inoculation. Heparinized blood was diluted 1:2 in heart infusion broth (HIB) (Difco Labs., Detroit, Mich.), and 0.5 ml was injected i.p. into each of 3 guinea pigs. Monkeys and guinea pigs were bled by cardiac puncture 24 days after inoculation, and sera agglutinins were determined by the method of Luoto (15), and CF antibodies were determined with a formalin-inactivated antigen of Nine Mile strain of *C. burneti* (Lederle Labs., New York, N.Y.).

[5] Taken by Mr. Alphonse G. Addison and SP-5 Curtis A. Allen, and interpreted by CPT Edward V. Staab, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

[6] Taken by SP-4 Arthur L. Self and interpreted by LTC Nelson R. Blemly, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

[7] Tests performed by Major Robert L. Taylor, Walter Reed Army Institute of Research, Washington, D. C.

B) When *Rickettsia rickettsi* was the test organism, monkeys were bled before inoculation and daily 3–11 days after inoculation; 2.5 ml heparinized blood was injected i.p. into each of 3 male guinea pigs. Infection was confirmed by the absence or presence of lesions and necrosis of scrotal skin, and by exsanguinating the guinea pigs by cardiac puncture 24 days after inoculation to determine CF antibody titers.[8]

Viruses: A) Viremia. Monkeys were bled before and after inoculation at intervals up to 14 days. Heparinized blood was diluted 1:2 in HIB (exceptions: with Rift Valley, blood was diluted 1:10; with yellow fever, blood was diluted 1:10 in HIB containing 20% egg yolk). From this suspension, each of 5 or 7 mice was inoculated i.p. or i.c., using 0.5-ml and 0.03-ml volumes, respectively. Mice were observed daily for 14 days for overt signs of infection for the particular virus.

B) Identification of virus. Mice injected for verification of viremia and controls that were moribund or died were aseptically necropsied. The brains from mice of the same cage were pooled, triturated, and made into a 20% suspension by weight. The suspension was centrifuged at 2000 rpm for 10 min, filtered through a Seitz filter, and frozen at −70°C until tested. The suspension was thawed quickly, and equal volumes of undiluted hyperimmune serum and 10-fold serial dilutions of tissue suspensions were mixed. After incubation at room temperature for 1 hr, each of 7 mice was inoculated i.c. with 0.03 ml or i.p. with 0.05 ml from each dilution of serum-virus mixture. Of the surviving mice, one-half were sacrificed and necropsied and the above procedure performed; the remaining mice were challenged with low doses of particular virus for protection tests.

C) Serum neutralization by mouse inoculation. Monkeys were bled before inoculation and 30 days after inoculation. All sera were inactivated at 56°C for 30 min. Standard diagnostic procedures (9) were used to compare the pre- and post-inoculation sera using constant serum and varying virus. Each of 7 mice was injected i.p. with 0.05-ml amounts of the serum-virus dilutions. Normal and hyperimmune sera controls were included in each test. The mice were observed for 21 days, at which time the neutralization index was computed by the method of Reed and Muench (22).

D) Tissue culture. Monkeys used in the *H. simiae* study were necropsied. Suspensions of spleen, cervical spinal cord, buccal mucosa, salivary gland, and lung were prepared by triturating the weighed section of tissue in medium 199 containing 10% calf serum, 500 units penicillin, and 500 μg streptomycin per ml and centrifuged at 2000 rpm for 10 min. Serial 10-fold dilutions were prepared and assayed in rabbit kidney (RK) monolayer cultures (Microbiological Associates, Inc., Bethesda, Md.) Each of 5 tubes of RK cultures was inoculated with 0.1 ml of the virus 10-fold dilutions. After virus adsorption for 30 min, 1 ml of medium 199 containing 2% calf serum, 200 units penicillin, and 200 μg streptomycin was added to each tube. Cultures were incubated at 37°C for 10 days and observed for cytopathogenic effect (CPE). Identification of *H. simiae* was confirmed by neutralization with *H. simiae* antiserum. The neutralization index was determined from sera of surviving monkeys collected 30 days after aerosol exposure by comparing neutralizing antibody with the preinoculation sera using the method described by Chappell (2) in RK cultures with 80 $TCID_{50}$. For the polio virus study, monkeys were bled before inoculation and 2, 4, 6, and 30 days after inoculation. Heparinized blood from each monkey was diluted 1:2 in Hanks balanced salt solution (BSS) and 0.2 ml inoculated on African green monkey kidney (MK) monolayer cultures (Microbiological Associates, Inc., Bethesda, Md.) The tubes were incubated at 37°C and observed 10 days for CPE. Monolayers exhibiting CPE were passaged and identified in MK cultures with specific polio virus rabbit

[8] CF tests were performed by Dr. B. L. Elisburg, Department of Rickettsial Diseases, Walter Reed Army Institute of Research, Washington, D. C.

antisera. Rectal swabs were taken of all monkeys 5, 9, 13, 16, 20, and 25 days after inoculation. The BSS-moistened cotton swabs were used, and the procedure of Craighead, Shelokov, and Brody (3) was followed, except the virus was isolated in MK cultures. Neutralization antibody was determined on pre- and 30-day post-inoculation blood samples. The tests were carried out in MK cultures by the method described by Schmidt and Lennette (24). All animals that died during the experiment were necropsied for histopathologic examination.[9]

E) Hemagglutination (HA) and hemagglutination-inhibition (HI) tests. Standard diagnostic HA tests using chicken red blood cells were followed. Confirmation of virus was determined by HI activity. For Newcastle virus, 10 HA units were used (20), and for influenza virus, 4 HA units were used (4). HI activity was determined using constant virus and varying the serum.

F) Identification of Newcastle disease virus (NDV). 1) Serum. A statistical sample of chickens was bled and determined to be NDV-free by HI assay of sera. All chickens were bled 14 and 24 days after inoculation. Sera were collected and frozen at −70°C, and upon completion of the experiment, HI activity was determined. 2) Twenty-four hours after inoculation, and for the next 7 days. tracheal swabs were taken from all chickens. Strict aseptic technique was observed so the experimenters would not infect the chickens. The swab was placed in tryptose broth (Difco Labs., Detroit, Mich.) containing 200 units penicillin and 200 μg streptomycin per ml. The eluted broth (0.2 ml) was inoculated intra-allantoically into each of 7, 10-day-old embroyonating eggs. Eggs dead within 24 hr were discarded. Allantoic fluids harvested from eggs that died within 5 days post-inoculation were individually tested for virus by HA test. Eggs demonstrating HA of the same tracheal swab were pooled and HI activity determined. 3) Chickens that died during the experiment were necropsied, and a sample of the brain was aseptically removed and triturated in tryptose broth containing 10,000 units penicillin and 5,000 μg streptomycin per ml. After centrifuging the suspensions at 2000 rpm for 10 min, 0.1 ml-volume of each supernatant fluid were inoculated intra-allantoically into 10, 10-day-old embroyonated eggs. HI testing of harvested allantoic fluids confirmed the absence or presence of infection.

G) Identification of influenza virus. A random sample of mice was bled to assay HI activity. At the conclusion of the experiment, mice were bled to determine from each serum HA and HI activity. Necropsies were performed on all mice and the lungs aseptically removed and triturated in HIB fortified with antibiotics. Infection was verified by HI tests.

5) Psittacosis agent. Before inoculating the monkeys, radiographs,[10] blood sedimentation rates, temperatures, and CF antibody titers[11] were performed, and a random sample of mice was bled for CF antibody titers. At daily intervals for 4 days after inoculation, all monkeys had radiographs, were bled, and the temperatures recorded. Blood sedimentation rates were recorded, and septicemia confirmed by diluting blood 1:5 in Sorensen's buffer containing 10% egg yolk (18) and injecting 0.2 ml i.p. into each of 5 mice. Twenty-eight days after inoculation all monkeys were bled for CF antibody response. Mice that died in the test for septicemia and used in cross infection experiments were necropsied. Smears from lungs, spleen, liver, and peritoneal exudate were stained with Macchiavello's and Giemsa stain and examined for elementary bodies. Suspensions made from the tissues were injected i.c. or i.p. into mice for further passage. Mice that survived were bled for CF antibody titers.

[9] Performed by Dr. Ruth L. Kirchstein, Chief, Laboratory of Pathology, National Institutes of Health, Bethesda, Maryland.
[10] Taken by SP-4 Arthur L. Self and interpreted by LTC Nelson R. Blemly, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.
[11] CF tests were performed by LTC Robert W. McKinney, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

TABLE 3

Summary of test results

Disease or Microorganism	Hosts	Inoculation Routes[a]	Air Wash (minutes)	Transmission to Cagemate Controls	Transmission to Adjacent Cage Controls	Maximum Hours after Caging During which Time Microorganisms were Recovered by Sampling Cage Exhaust Air			
						Regular Air Wash		Ruffling Air Wash	
						Cage 1[b]	Cage 2[c]	Cage 1[b]	Cage 2[c]
Bacillus anthracis	Monkeys	w.b.	25	Yes[d, e]	Yes[e]	88	82	4	2
		h.o.	25	Yes[e, f]	Yes[e]	64	60	2	0
		i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
	Guinea pigs	i.m., s.c.	—	No	No	—	—	—	—
Brucella suis	Monkeys	w.b.	15	Yes[e]	Yes[e]	30	24	2	0
		h.o.	15	Yes[e]	Yes[e]	18	10	1	0
		i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
	Mice	i.m., i.n., i.p., s.c.	—	No	No	—	—	—	—
Coxiella burneti	Monkeys	w.b.	15	Yes[e]	Yes[e]	88	72	2	0
		h.o.	15	Yes[e]	Yes[e]	80	56	1	0
		i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
	Guinea pigs	h.o.	15	Yes	Yes[e, h]	—	—	—	—
		i.m., i.n., i.p., s.c.	—	No	No	—	—	—	—
Herpesvirus simiae	Monkeys	w.b.	15	Yes	Yes	3	1	0	0
		h.o.	15	Yes	Yes[e]	2	0	0	0
Histoplasma capsulatum	Monkeys	w.b.	25	Yes[e, f]	Yes[e]	54	50	4	1
		h.o.	25	Yes[e, f]	Yes[e]	36	28	1	0
		i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
	Guinea pigs	w.b.	15	No	No	—	—	—	—
		i.m., i.p., s.c.	—	No	No	—	—	—	—
	Mice	i.m., i.n., i.p., s.c.	—	No	No	—	—	—	—
Influenza	Mice	i.m., s.c.	—	No	No	—	—	—	—
		h.o.	15	Yes	No	—	—	—	—
		i.n., i.p.	—	Yes	No	—	—	—	—
Japanese B encephalitis	Monkeys	w.b., h.o.	15	No	No	6	1	0	0
	Mice	w.b., h.o.	15	No	No	—	—	—	—
Mycobacterium tuberculosis	Monkeys	w.b.	15	Yes	Yes	144	144	48	40
		h.o.	15	Yes	Yes	120	112	32	30
	Guinea pigs	h.o.	15	Yes	Yes[e, h]	—	—	—	—
	Mice	w.b., h.o.	15	Yes	Yes[e]	—	—	—	—
		i.m., i.p.	—	Yes	No	—	—	—	—
Newcastle disease virus	Chickens	i.t.	—	Yes	Yes[e, h]	—	—	—	—
Pasteurella pestis	Guinea pigs	i.m., i.p.	—	No	No	—	—	—	—
		i.n.	—	Yes	No	—	—	—	—
	Mice	w.b.	15	Yes	No	—	—	—	—
		i.n., i.p.	—	Yes	No	—	—	—	—
		i.m.	—	No	No	—	—	—	—

Disease or Microorganism	Hosts	Inoculation Routes[a]	Air Wash (minutes)	Transmission to Cagemate Controls	Transmission to Adjacent Cage Controls	Maximum Hours after Caging During which Time Microorganisms were Recovered by Sampling Cage Exhaust Air			
						Regular Air Wash		Ruffling Air Wash	
						Cage 1[b]	Cage 2[c]	Cage 1[b]	Cage 2[c]
Pasteurella tularensis	Monkeys	w.b., h.o.	15	Yes[d]	No	2	0	0	0
		i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
	Guinea pigs	i.m., i.n.	—	No	No	—	—	—	—
Polio virus	Monkeys	w.b.	15	No	No	34	26	2	0
		h.o.	15	No	No	26	14	0	0
		i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
Psittacosis	Monkeys	w.b.	15	Yes[e]	Yes[e]	40	36	1	0
		h.o.	15	Yes[e]	Yes[e]	24	24	0	0
		i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
	Mice	i.c., i.p.	—	No	No	—	—	—	—
Rickettsia rickettsi	Monkeys	w.b.	15	Yes[e, i]	Yes[e]	24	18	2	0
		h.o.	15	Yes[e, i]	Yes[e]	16	10	1	0
		i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
	Guinea pigs	i.p., s.c.	—	No	No	—	—	—	—
Rift Valley fever	Monkeys	w.b.	15	Yes[e, j]	Yes[e]	8	2	0	0
		h.o.	15	Yes[e, j]	Yes[e]	8	0	0	0
		i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
	Mice	i.c., i.p.	—	No	No	—	—	—	—
St. Louis encephalitis	Monkeys	i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
	Mice	i.c., i.p.	—	No	No	—	—	—	—
Venezuelan equine encephalitis	Monkeys	w.b.	15	Yes[e, i]	Yes[e]	48	40	2	1
		w.b.	10[k]	Yes	No	24	12	—	—
		h.o.	15	Yes[e, i]	No	36	32	1	0
		i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
	Guinea pigs	h.o.	15	Yes	No	—	—	—	—
		i.m., i.p.	—	No	No	—	—	—	—
	Mice	w.b.	15	Yes	No	—	—	—	—
		i.c., i.p.	—	Yes[l]	No	—	—	—	—
Yellow fever	Monkeys	w.b.	15	Yes[e]	Yes[e]	32	24	0	0
		h.o.	15	Yes[e]	Yes[e]	16	12	0	0
		i.m., i.p., i.v., s.c.	—	No	—	—	—	—	—
	Mice	w.b.	15	Yes	No	—	—	—	—
		i.n., i.p.	—	No	No	—	—	—	—

a. w.b. = whole body exposure; h.o. = head only exposure; i.c. = intracerebral; i.m. = intramuscular; i.n. = intranasal; i.p. = intraperitoneal; i.t. = intratracheal; i.v. = intravenous; s.c. = subcutaneous; - = no test.

b. Exposed monkey and cagemate control.

c. Adjacent cage control.

d. Monkeys, when placed with exposed animals, 24, 48, and 72 hours after exposure, contracted the disease; however, after 96 hours there was no transmission.

e. No transmission when the exposed monkey was air-washed for 10 minutes with ruffling of fur followed by a 5 minute normal air-wash.

f. Monkeys, when placed with exposed animals 24 and 48 hours after exposure, contracted the disease; however, after 72 hours there was no transmission.

g. No transmission to animals in adjacent cages when ultraviolet irradiation was emitted across tops of cages.

h. No transmission when high-efficiency air filters were installed on top of each cage.

i. Monkeys, when placed with exposed animals 24 hours after exposure, contracted the disease; however, after 48 hours there was no transmission.

j. Monkeys placed with exposed animals 24 hours after exposure did not contract the disease.

k. After air washing, the monkeys were wiped with a towel moistened with 2% quaternary ammonium compound.

l. No transmission of disease to animals housed in wire-bottomed cages.

Results

The results of these studies are summarized in Table 3. Although this table is long and appears to be complex, it incorporates the results of transmission studies with 1) 1,016 monkeys, of which 268 were exposed to microbial aerosols, 268 were cagemate controls, 192 were adjacent cage controls, and 144 were injected parenterally and caged with 144 controls; 2) 2,194 guinea pigs, of which 1,097 were infected, 575 were cagemate controls, and 522 served as adjacent cage controls; 3) 8,360 mice, of which 4,620 were infected, 1,760 were cagemate controls, and 1,980 were adjacent cage controls; and 4) 845 chickens, of which 350 were infected, 195 were cagemate controls, and 300 were adjacent cage controls. To account for these 12,415 animals, a separate table could have been used for each species but interpretation would then have been difficult. An an aid for the reader in understanding this table and to avoid repetition, a detailed report is presented for *B. anthracis*. Only a brief summary of the results of the remaining 17 tests of pathogens is included here unless a special test or result required more detailed information.

Bacillus anthracis: When monkeys were given a respiratory inoculum by exposing either the whole-body or only the head to an aerosol, and were air-washed for 25 min immediately before caging with control monkeys, they, their cagemate controls, and adjacent cage controls contracted anthrax. When cagemate controls were placed with whole-body exposed monkeys 24, 48, 72, and 96 hr after exposure, only the controls put in cages at 96 hr did not contract anthrax. When the experiment was repeated using head-only exposed monkeys, only the controls put in the cages 72 and 96 hr after exposure did not contract anthrax.

B. anthracis was recovered by air samples from the air exhaust duct from the cages for 88, 82, 64, or 60 hr, depending upon the method of inoculation. When the aerosol-challenged monkeys were air-washed by ruffling the fur, although they contracted anthrax, there was no transmission to the cagemate or adjoining cage controls. Air sampling in the exhaust air ducts indicated that ruffling the fur reduced the time during which *B. anthracis* was eliminated from the fur; for instance, from 88 hr to 2 hr.

Monkeys injected i.m., i.p., i.v., or s.c., and guinea pigs injected i.m. or s.c. contracted anthrax but did not transmit the disease to control animals. Whether the guinea pigs walked on a screen above the litter or walked in the litter did not affect the results.

Coxiella burneti: Monkeys exposed by respiratory exposure transmitted the disease to control monkeys, but this was cross contamination because ruffling the fur of exposed monkeys eliminated infection of controls.

Guinea pigs infected by head-only aerosol exposure and then air-washed transmitted the disease not only to 14 of 20 cagemate controls, but to 9 of 18 adjacent cage controls. This adjacent cage transmission was eliminated either when ultraviolet irradiation with an average intensity of 350 μ watts/sq cm over each cage was emitted across the cages, or when 50 FG air filters were attached to the cages. However, cagemate transmission of disease within each cage still occurred even when ultraviolet irradiation or 50 FG air filters were used.

Herpesvirus simiae: When monkeys were given a respiratory inoculum and then air-washed before caging, they, their cagemate, and adjoining cage control monkeys contracted the infection. Animals given a whole-body respiratory inoculum and fur ruffled transmitted the disease to 2 of 3 cagemates and 2 of 3 adjacent cage control monkeys. When monkeys were exposed head-only to the aerosol and then air-washed by ruffling, 1 of 3 cagemates became infected, but the controls in the adjoining cages did not contract the disease.

Influenza virus: Transmission of disease only occurred in cagemate controls when mice were inoculated by head-only aerosol route (19 of 40), i.n. (11 of 40), or i.p. (6 of 40); however, adjacent cage controls did

not become infected.

Japanese B. encephalitis virus: No cross contamination or cross infection.

Mycobacterium tuberculosis: Air-washing, whether it was for 15 min or by ruffling the fur, did not prevent the spread of tuberculosis to the cagemate and adjacent cage control monkeys. The number of controls contracting the disease was less when the ruffling procedure was used, and *M. tuberculosis* was recovered less frequently from the cage exhaust air after ruffling.

Guinea pigs infected by head-only exposure and then air-washed transmitted the disease not only to 18 of 20 cagemate controls, but to 11 of 18 adjacent cage controls. When ultraviolet irradiation with an average intensity of 350 μ watts/sq cm was emitted across the cages, 11 of 13 cagemate controls contracted tuberculosis. The addition of 50 FG air filters also prevented the adjacent cage controls from contracting tuberculosis, although 10 of 13 cagemate controls became infected.

Mice inoculated by the respiratory route and air-washed became infected, as did 15 of 19 cagemate controls and 8 of 25 adjacent cage control mice. Ultraviolet irradiation emitted across the tops of the cages did not prevent 9 of 18 cagemate control mice from contracting tuberculosis but did prevent adjacent cage control mice from contracting the disease. Mice injected i.m. or i.p. contracted the disease, as did cagemate control mice (i.m. 23 of 40, i.p. 29 of 40); however, adjacent cage controls remained free from tuberculosis.

Newcastle disease virus: Cagemate control chickens became infected regardless of the number of i.t. inoculated chickens in the cage. Various caging arrangements were tested by eliminating cagemate controls and using only adjacent cage controls. Results of diagnostic tests revealed that controls on wire still became infected, but on the average 1 day later than those housed directly on litter. Figure 3 is a schematic arrangement of the cages that will aid the reader in understanding the next series of experiments. Inoculated chickens were located in cages A, E, G, J, L, and O; controls were in the remaining cages. The top of cages A, E, G, and I through P were covered with 50 FG air filters. Ultraviolet irradiation was emitted across each cage. The inoculated chickens developed Newcastle disease, but there was no transmission of disease to any control chicken.

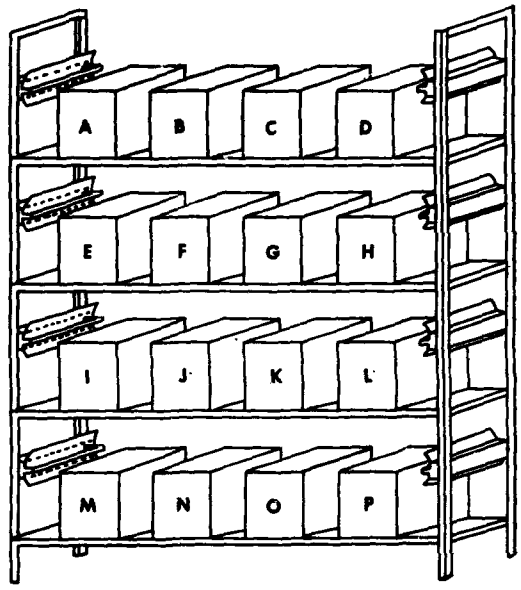

Fig. 3. *Schematic caging arrangement for chickens. U. S. Army photograph.*

Experiments were designed to evaluate ultraviolet and 50 FG air filters separately. On 1 cage rack ultraviolet irradiation was emitted across all cages; and on the other cage rack, all cages were covered with air filters. Inoculated chickens were located as in the previous experiment. Diagnostic tests revealed that only the inoculated chickens contracted the disease, and there was no transmission.

A combination experiment was designed. Inoculated chickens were located in cages A&C, F&H, and I&K. Control chickens in cages E and G had air filter cagetop covers. On the shelf where I through L were located, ultraviolet irradiation was used. On the other cage racks, the position of the ultraviolet shelf or 50 FG filter shelf was changed to assure that shelf location was not

a factor. On all cage racks there was no transmission of NDV where air filter tops or ultraviolet irradiation was employed. Yet, transmission occurred from cage-to-cage on all shelves where no filter or ultraviolet was used.

Pasteurella pestis: Guinea pigs inoculated i.n. developed the disease, and infection was transmitted to 5 of 13 cagemate controls. There was no evidence of transmission to adjacent cage controls.

Mice exposed whole-body to a respiratory inoculum and air-washed became infected, as did 7 of 19 cagemate control mice. Adjacent cage control mice remained free from disease. Mice injected i.n. or i.p. became infected and transmitted the disease to 11 of 40 and 13 of 40 cagemate control mice, but not to control mice in adjacent cages.

Pasteurella tularensis: When monkeys were exposed either by whole-body or only the head to an aerosol, and air-washed before caging, both they and their cagemate controls contracted tularemia, but the control monkeys in the adjoining air-duct-connected cages did not become infected. Ruffling the fur eliminated transmission to cagemate controls.

Polio virus: No cross contamintion or cross infection.

St. Louis encephalitis virus: No transmission of disease.

Venezuelan equine encephalitis virus: When the monkeys received a head-only challenge and a 15-min-air-wash, or received a whole-body challenge and then a 10-min-air-wash followed by a wipe of the entire body by a towel moistened with 2% quaternary ammonium compound, only the cagemate controls became infected; the monkeys in the air-duct-connected 2nd cage did not.

Air-ruffling the fur of aerosol-exposed monkeys prevented transmission of the disease to cagemates or monkeys in air-duct-connected cages and reduced the number of hours that air sampling recovered the virus.

Guinea pigs given a respiratory inoculum by exposing only the head to an aerosol and then air-washed transmitted the disease to 7 of 20 cagemate controls, but the adjacent cage controls remained free from disease.

Mice exposed (whole-body) to a respiratory inoculum and air-washed became infected, as did 9 of 19 cagemate controls, but adjacent cage controls remained free from disease. Mice injected i.c. or i.p. became infected, as did 9 of 80 and 15 of 80 cagemate controls when they walked in cage litter, but adjacent cage controls did not contract the disease. Mice that walked on wire screen did not transmit infection to cagemate or adjacent cage control mice.

Yellow fever virus: Air-ruffling the fur of the aerosol-exposed monkeys prevented transmission of the disease to cagemates or monkeys in air-duct-connected 2nd cages, and air samples did not recover the virus.

Mice inoculated by whole-body exposure to virus and air-washed became infected, as did 2 of 19 cagemate controls, but adjacent cage controls remained free from disease.

Results with *Brucella suis, H. capsulatum*, psittacosis agent, *R. rickettsi*, and Rift Valley fever virus were similar. Normal air-wash of monkeys exposed to microbial aerosols did not prevent cagemate and adjacent cage controls from contracting the disease. The secondary aerosol from microorganisms entrained on the fur was reduced by the ruffling technique so that cross contamination was eliminated. Other animals infected by these pathogens did not infect control animals.

Discussion

Detailed examination of the results show that whole-body or head-only microbial aerosol challenge caused cagemate infection, and presumably danger, not only to the experimenter from animal contact, but to the experiment by augmenting the dose. This occurred even though the exposed animals had been in the airstream for 10–15 min after challenge before the cagemates were placed with them. This applies to the following

leased from the fur or skin before the exposed animals become ill, and *cross infection,* by which diseased animals infect cagemates by urine, feces, saliva, or droplets (14).

Parentally infected animals do not infect normal cagemates in many instances in which the microorganism is known to cause frequent or serious human illness. In our experiments with monkeys, there were 11 human pathogens tested, none of which caused cagemate infection. The caging arrangements discouraged cross infection, because excreta fell to waste-collecting pans, from which monkeys could not reclaim feces or food. Fifteen complete air changes per hr in the animal room also were helpful.

Guinea pigs or mice infected by parenteral inoculation usually do not transmit the disease to cagemates. However, when the disease involves the respiratory system, intracage transmission may occur (influenza, plague, tuberculosis). Attention to mice parenterally injected with Venezuelan equine encephalitis virus reveals very interesting data. Mice injected i.c. or i.p. and housed so that urine and feces dropped from the cage did not transmit infection to control cagemates, but when the mice were housed in solid-bottomed cages containing litter, infection was transmitted to control cagemates. Rodents excrete all test microorganisms in Table 3 that mice were inoculated with except psittacosis agent, St. Louis encephalitis, Rift Valley fever, and yellow fever viruses (28). Yet, unless the microorganisms had a proclivity for respiratory tissue, transmission occurred only in mice parenterally injected with Venezuelan equine encephalitis. The mechanisms of host susceptibility are very obscure, and daily inhaled subinfective doses may or may not be neutralized by animal body defenses, a factor determining infection.

Ultraviolet irradiation or filter tops on cages are useful in preventing infection of normal animals in adjacent cages. This was shown by test with tuberculosis in guinea pigs and mice, *C. burneti* in guinea pigs, and NDV in chickens. Others have had similar experience with ultraviolet irradiation (10, 17, 21, 23) and filter tops (13, 25).

Cross infection among experimental animals is affected by many variables, such as the microorganism, animal, method of inoculation, amount of inoculum, caging, feeding and watering, treatment of exposed animals after inoculation in regard to air washing or other means to remove inoculum from the skin and hair, cannibalism, use of ultraviolet irradiation, and use of air filters on cages. Moreover, there can be unforeseen and unrecognized additional significant variables. From the present studies, and from incorporation of data in a tabular summary (28), certain general recommendations emerge, as follows:

1) After microbial aerosol respiratory challenge, to avoid augmentation of the challenge dose by shake-off of organisms from the animal fur, it is a good practice to place each animal in a separate cage or to cage together only those animals that receive the same dosage.

2) To protect personnel from release of organisms from the fur after microbial aerosol respiratory challenge of animals, it is desirable that the animals initially be placed in a closed cage, preferably ventilated. Even when normal cagemates are not infected, organisms can still be recovered from the air of the cage.

3) Safe removal of aerosol-challenged animals from a closed cage and transfer to more simple caging arrangements depends only partly upon the time required for the microorganisms on the fur to die or be removed by the cage ventilation. There is a significant variation in this time, depending upon the microorganism, challenge, and post-exposure treatment. Moreover, the extent of excretion in urine and feces sometimes is very important to personnel safety, regardless of the method of animal inoculation.

4) If feasible, animals exposed to aerosols should be air-washed by ruffling the fur, as this technique greatly reduces cross contamination.

5) In parenterally injected animals, when infection of cagemate controls complicates

combinations of animals and infectious agents: monkey – *B. anthracis, Br. suis, C. burneti, H. simiae, H. capsulatum, M. tuberculosis, P. tularensis,* psittacosis agent, *R. rickettsi,* and the viruses of Rift Valley fever, Venezuelan equine encephalitis, and yellow fever; guinea pig – *C. burneti, M. tuberculosis,* and Venezuelan equine encephalitis; mouse – *M. tuberculosis, P. pestis,* and the viruses of influenza, Venezuelan equine encephalitis, and yellow fever. There were some combinations in which there was no cross infection: monkey – Japanese B encephalitis and polio viruses; guinea pig – *H. capsulatum;* mouse – Japanese B encephalitis virus. Possibly the air-wash of aerosol-challenged animals accounted for the exception of monkey-polio virus, because i.n. or oral instillation has been reported (28) associated with infection of normal cagemates.

As a general rule, and within the limits of the experimental results available, if a cagemate was infected after being placed with an animal challenged by whole-body aerosol exposure, the same occurred after head-only aerosol exposure. Two exceptions are recorded: monkey – *C. immitis* and guinea pig – *P. tularensis* (28). The air wash may have removed enough inoculum from the head to prevent infection of the cagemate. In these 2 combinations of animal and disease, no method of challenge except whole-body exposure caused cagemate infection. Similarly, normal animals in closely adjacent or duct-connected cages to aerosol-exposed animals usually were infected; exceptions occurred when monkeys were exposed whole-body or head-only to *P. tularensis* and head-only to Venezuelan equine encephalitis virus; guinea pigs exposed head-only to Venezuelan equine encephalitis virus; and mice exposed head-only to influenza and whole-body to *P. pestis* and the viruses of Venezuelan equine encephalitis and yellow fever.

The usual post-exposure air-washing techniques applied to aerosol-exposed animals were not reliable methods of preventing infection of normal cagemates. Apparent successes were few, and proof was incomplete. Air-wash followed by wiping the animal with a towel moistened with 2% quaternary ammonium disinfectant did not prevent cagemate infection in the case of monkey exposed to dry arthrospores of *C. immitis* (14), but it might explain failure to infect in an adjacent cage in the case of monkey exposed to Venezuelan equine encephalitis virus.

However, use of a manipulated forceful jet of air to ruffle the fur for 10 min plus a 5-min normal air-wash was outstandingly successful where the usual air-wash had failed. This prevented infection of normal monkeys caged with monkeys whose entire bodies had been exposed to aerosols of *B. anthracis, Br. suis, C. burneti, H. capsulatum, P. tularensis,* psittacosis agent, *R. rickettsi,* and Rift Valley fever, Venezuelan equine encephalitis and yellow fever viruses. The method was not successful with *H. simiae* or *M. tuberculosis* aerosol exposures. These 2 organisms, therefore, must be regarded as most dangerous.

When exploring the reasons for transmission of the test microorganisms after aerosol challenge, many variables must be considered. In this study, the monkeys were in cages constructed so that urine and feces dropped from the cage floor to waste-collecting pans out of reach of the monkeys. Partially eaten food possibly contaminated by saliva that was dropped by the monkeys also fell out of reach. Viable microorganisms that might have been excreted or microorganisms on contaminated food were removed from the cages by constant air changes. Monkeys did not walk in bedding. When aerosol-challenged monkeys were air-washed for 15 or 25 min, of 276 cagemate and adjacent cage controls, 65.1% became infected. However, forceful ruffling of the fur by manipulating an air hose did clean the fur to a point where only 7.3% of 178 cagemate and adjacent cage controls contracted the disease, and this occurred only when *H. simiae* or *M. tuberculosis* was the test microorganism. These data demonstrate the difference between *cross contamination,* by which cagemates are infected by microorganisms re-

interpretation of experimental results, placing the animals on wire screen that permits urine and feces to drop through may reduce the cross infection. Evidence on this point was confined to the results wherein mice were injected intraperitoneally or intracranially with VEE.

6) Ultraviolet irradiation or filter tops on cages of infected animals prevents infection of normal animals in adjacent cages.

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